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Abstracts

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STUDY OF RAINBOW TROUT *(ONCORHYNCHUS MYKISS)* GONADS RECEPTIVENESS TO ESTRADIOL AND ANDROGEN : ISOLATION AND USE OF HOMOLOGOUS cDNA PROBES

M-L MALEJAC(1), Y. VALOTAIRE(2), A. FOSTIER(1) and C. ATTEKE(3)
(1)Institut National de la Recherche Agronomique, Laboratoire de Physiologie des poissons, Université de Rennes, (2)Laboratoire de Biologie Moléculaire, (3)Laboratoire de Physiologie des régulations,
Campus de Beaulieu 35042 Rennes, CEDEX, France

Numerous experiments demonstrated that sexual steroids were able to inverse sex in fish when administered *in vivo*. However, there is no direct evidence showing that embryonic gonads are themselves sensitive to sexual steroids. Our purpose was to check rainbow trout fry gonads for the presence of estradiol and androgen receptor messenger RNA. Total and Poly (A+) RNA were prepared from gonads sampled during sexual differentiation of normal and female monosexual cohorts of rainbow trout. An homologous estradiol receptor cDNA containing steroid and DNA binding domains was used for Northern blot analysis. Besides, a rat androgen receptor cDNA containing both steroid and DNA binding domains was used in order to achieve the homologous probe as no positive signal could be obtained by hybridizing the gonads Poly (A+) mRNA with the heterologous probe. Thus we performed Northern blot hybridizations with total and messenger RNAs from various rainbow trout tissues in order to check an appropriate cDNA library.

Concerning androgen receptor, slight positive signals were suspected in Northern blots performed with mRNA isolated from the *proximal pars distalis* (PPD) of the pituitary. A cDNA library specific for this tissue was screened allowing us to detect 23 positive clones. Purification and sequencing are now in progress in order to isolate and characterize an homologous androgen receptor cDNA for rainbow trout. It will be used to check for the presence of mRNA androgen receptor in embryonic gonads of the trout.

Concerning Estradiol receptor mRNA, several stages were studied : 87-93, 94-98, 110-118, 148 and 180 days post fertilization (rearing temperature = 13.5°C). The estradiol receptor mRNA was only detected in female and male rainbow trout gonads sampled at 180 days postfertilization. The lack of detectable positive signals with the other stages in Northern blot analysis may be due to the small quantity of available mRNA (0.6 to 8 μg/100 gonads).

So we develop an *in situ* hybridization technique and we first studied female and male gonads at 180 days post fertilization. The gonads, fixed in paraformaldehyde were embedded in paraffin. After pretreatment, the sections were hybridized with a 350 pb PstI-SphI fragment from the E domain of the rainbow trout cDNA estradiol receptor, labelled with (^{35}S)-UTP. At this time, no clearly localized positive signal could be detected in gonads tissues sections. This result may be due to the fact that many cells express the estradiol receptor mRNA but at a low basal level making the detection of specific signal difficult, even if this technique is very sensitive.

However our first results did not demonstrate precocious estradiol receptor in undifferenciated ovaries. They are in agreement with the hypothesis that estradiol is not involved during the first stages of gonadal differentiation.